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Chlorinated Ethene Half-Velocity Coefficients (K_S) for Reductive Dehalogenation

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Biological reduction of the chlorinated solvents tetrachloroethene (PCE) and trichloroethene (TCE) completely to ethene is of interest for engineered or intrinsic destruction of these prevalent groundwater contaminants. However, the transformations are frequently not complete, leading to the production of vinyl chloride (VC), a more hazardous compound. Factors affecting the relative rates of destruction of the solvents and their intermediate products are thus of interest. The maximum degradation rates (kX) and half-velocity coefficients (K_S) for these chlorinated ethenes used as electron acceptors in reductive dehalogenation with hydrogen in excess were examined using an enrichment culture grown on benzoate, hydrogen, and PCE. Initial dehalogenation rates were measured at various chlorinated ethene concentrations in batch studies. With 38 mg/L volatile suspended solids of this culture, the kX and 95% confidence intervals for PCE, TCE, *cis*-dichloroethene (cDCE), and VC at 25 °C were found to be 77 ± 5, 59 ± 11, 14 ± 3, and 13 ± 3 μM/day with K_S values of 0.11 ± 0.04, 1.4 ± 0.9, 3.3 ± 2.2, and 2.6 ± 1.9 μM, respectively. The lower maximum transformation rates and higher K_S values for cDCE and VC partly explain why incomplete transformation of PCE and TCE often occurs in the field.

Introduction

Chlorinated aliphatic hydrocarbons (CAHs) such as tetrachloroethene (perchloroethene; PCE) and trichloroethene (TCE) are among the most common organic groundwater pollutants in the United States (*1*). Although PCE is not degraded under aerobic conditions, field studies and laboratory evidence for dechlorination in the absence of oxygen have been widely reported (*2*−*5*). For anaerobic dechlorination, the CAHs studied serve as electron acceptors (*6*−*9*). Thus, for dechlorination to occur, an electron donor must be provided, and numerous organic substrates have been shown to serve this function, perhaps by being fermented with the formation of hydrogen that serves as the primary electron donor for dehalogenation (*5*, *10*−*12*). Given an appropriate electron donor under anaerobic conditions, PCE is reductively dechlorinated stepwise through TCE, *cis*-dichloroethene (cDCE), vinyl chloride (VC), and then to ethene. The intermediate VC is a known human carcinogen, and thus its formation and persistence is of special concern.

The effects of varying concentrations of the electron donor, hydrogen, have been documented (*13*, *14*), but the influence of acceptor (CAH) concentrations has received little study. It is generally thought that under reducing conditions the more chlorinated ethenes are dehalogenated faster than the less chlorinated ones, resulting in the most toxic intermediate, VC, being degraded the slowest (*15*). This study aims to further investigate factors affecting the maximum dechlorination rates and half-velocity coefficients (K_S) for chlorinated ethenes being reductively dehalogenated.

Materials and Methods

Culture. An anaerobic mixed culture capable of completely dechlorinating PCE to ethene was seeded with aquifer material from a PCE-contaminated site in Victoria, TX. This site had been bioremediated under sulfate-reducing conditions through the addition of benzoate (*16*). The culture was originally enriched using site groundwater to which was added 60 mg/L sodium benzoate, 2 mmol hydrogen, and 30 μM PCE. Benzoate and hydrogen were used in excess to ensure that complete dechlorination was obtained. Site groundwater was later replaced with a basic mineral medium (*17*) containing 20 mg/L yeast extract as well as the benzoate, hydrogen, and PCE. With this composition, PCE was essentially the only external electron acceptor present. After several transfers using 10^{-3} dilutions, the resulting culture was used for an anaerobic continuously stirred tank reactor (CSTR) that was operated at 25 °C and continuously fed 100 mL/day basic mineral media containing 0.7 mM PCE, 1.74 mM benzoate, and 20 mg/L yeast extract, yielding a 36-day detention time. Additionally, 1 mmol of hydrogen was added every 2 days to the headspace of the 4.3-L CSTR, which had a 3.6-L liquid volume. PCE was dechlorinated to ethene in the reactor, and benzoate was completely consumed, producing acetate and methane (*17*). After several months of operation, effluent from this CSTR was used as seed culture for batch kinetic studies. Volatile suspended solids (VSS) in the reactor during these experiments remained at 58 ± 2 mg/L, and the pH was 7.1 ± 0.2.

Batch Studies. Batch studies were conducted at 25 °C by adding 76 mL of basic mineral media, 4.4 mg of yeast extract, and 13 mg of sodium benzoate into 254-mL bottles capped with Mininert valves (Alltech, Deerfield, IL) in an anaerobic glovebox that was filled with 80% nitrogen, 10% carbon dioxide, and 10% hydrogen. CAHs were then added from stock solutions. After being shaken horizontally in the glovebox on a shaker table set at 1000 rpm, the bottles were seeded with 144 mL of fresh culture from the CSTR, resulting in a VSS concentration of 38 mg/L. Bottles with different initial concentrations of each chlorinated ethene were operated simultaneously on the shaker table. Initial dehalogenation rates were measured, generally over a period of less than 1 h before subsequent dehalogenation products rose to concentrations that otherwise might have affected the rates. Control bottles showed no significant losses of the CAHs during this time. Dechlorination progress and rates were measured by headspace analysis with initial and final aqueous samples also being taken for comparison of aqueous to headspace chlorinated ethene concentrations. Headspace samples were also taken for hydrogen analyses.

The ratio of headspace to aqueous CAH concentrations at the start and finish of these experiments is summarized in Table 1. The ratios compare well to their reported dimensionless Henry's constants, and no significant change was observed in this ratio as the concentration decreased during these experiments, indicating good mass transfer between gas and liquid phases for these compounds in these vigorously shaken bottles. During all batch experiments, headspace hydrogen concentrations ranged between 6 and

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TABLE 1. Ratio of Headspace (μM) to Aqueous (μM) Concentration for Each CAH Measured at the Beginning and End of Batch Experiments and Reported Dimensionless Henry's Coefficients at 25 °C[a]

	ratio of headspace to aqueous concentrations		dimensionless Henry's law coeff (*29*)
	initial	final	
PCE	0.71 ± 0.07	0.73 ± 0.05	0.72
TCE	0.40 ± 0.05	0.41 ± 0.04	0.39
cDCE	0.19 ± 0.03	0.18 ± 0.04	0.15
VC	1.03 ± 0.10	1.04 ± 0.09	1.07

[a] Although CAH concentrations decreased during the experiments, the ratios did not change significantly, suggesting good mass transfer between gas and liquid phases during the experiment.

7 kPa. This would result in equilibrium liquid concentrations that are 2 or 3 orders of magnitude greater than the K_S values for hydrogen reported by Smatlak et al. (*13*) and Ballapragada et al. (*14*) and so were not expected to be rate limiting. To further check for a dechlorination rate dependence on hydrogen concentrations, 6 μM PCE was added to batch bottles with headspace hydrogen concentrations ranging from 0.6 to 15 kPa. No significant change in dechlorination rate was observed through this range of hydrogen concentrations. Additionally, initial dechlorination rates were not observed to change by increasing yeast extract concentrations up to 200 mg/L nor with benzoate concentrations up to 1.7 mM. With good mass transfer and the electron donors in excess, true intrinsic K_S values for the chlorinated compounds could thus be determined using headspace measurements.

Analytical Methods. The 0.25-mL headspace samples were used for the measurement of hydrogen, ethene, ethane, methane, PCE, TCE, cDCE, and VC. Chlorinated ethenes were measured with a Fractovap 2900 series gas chromatograph (Carlo Erba Strumentazione, Milan, Italy) equipped with a 10.2 eV photoionization detector and a DB624 column (3 μm film, 30 m × 0.53 mm, J&W Scientific) that was operated isothermally at 60 °C for PCE and TCE or at 40 °C for cDCE and VC rate experiments. Methane, ethene, and ethane were analyzed with a gas chromatograph (Hewlett-Packard model 5730A, Palo Alto, CA) operated isothermally at 90 °C with a packed 60/80 Carbosieve G column (5 ft by 0.125 in.; Supelco, Bellefonte, PA) and a flame ionization detector. Hydrogen concentrations were measured with a reduction gas detector (RGD2; Trace Analytical, Menlo Park, CA).

The 200-μL aqueous samples were analyzed for chlorinated ethene concentrations using a Tekmar model 4000 dynamic headspace concentrator with a model ALS automatic laboratory sampler to purge-and-trap the CAHs followed by separation with an HP 5890 series II gas chromatograph and quantification with a Tracor 700A HALL electrolytic conductivity detector. The samples were purged at 30 °C for 10 min and desorbed for 2.5 min at 110 °C followed by baking at 150 °C for 2 min. The gas chromatograph was set at 45 °C for 10 min, ramped at 70 °C/min to 150 °C, and held for 4.3 min. In all cases, identification and quantification was by comparison with external standards.

Chemicals and Gases. Chemicals for the media along with the chlorinated compounds PCE, TCE, and cDCE in high purity were obtained from Sigma and Aldrich (Milwaukee, WI). VC (>99.5% pure) was obtained from Fluka (Milwaukee, WI). Yeast extract was obtained from Difco Laboratories. Standards for hydrogen, methane, ethene, and ethane in nitrogen were obtained from Alltech (Deerfield, IL).

Biomass Determination. With the mixed culture, biomass in the bottles was measured with each experiment as volatile suspended solids (VSS) according to standard methods (*18*) using a GF/F filter and triplicate controls.

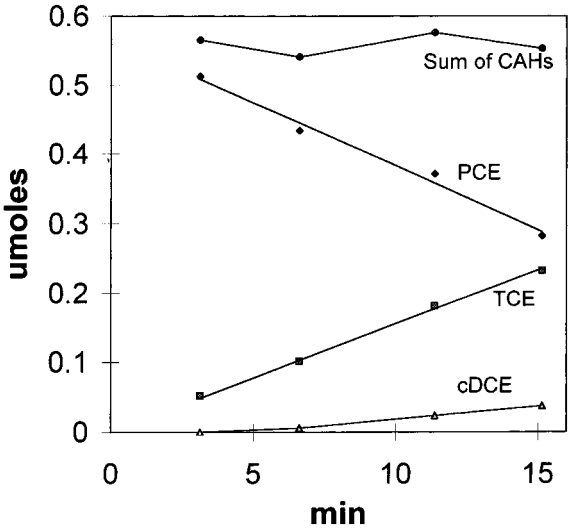

FIGURE 1. Typical dechlorination results from a batch experiment, in this case for PCE. Reported total CAH mass in the bottle was computed from headspace measurements using published Henry's constants (*29*). A linear fit of the PCE data points was used to find the initial dehalogenation rate, and the first measured data point was used as the initial concentration.

Data Analysis. The rate of dehalogenation of a given CAH may be influenced by a number of factors, including the concentrations of both the electron donor and acceptor (CAH), potential toxicity of the CAH itself, and competitive inhibition. However, in all experiments reported herein, the concentrations of CAHs were kept below toxic levels (*19*), and the donor (hydrogen) was kept in excess. Potential product competitive inhibition was avoided by measuring initial dehalogenation rates before product concentration became an issue. Thus, experimental conditions reduced the predicted dehalogenation rate to the simple form:

$$-\frac{dS}{dt} = \frac{kXS}{S + K_S} \quad (1)$$

where S is the CAH concentration, K_S is its half-velocity coefficient, k is the maximum CAH dechlorination rate per unit biomass, and X is the biomass concentration. Biomass concentrations remained constant during these short experiments. Initial dehalogenation rates ($-dS/dt$) were measured over a range of chlorinated ethene concentrations (S) for each of the CAHs. Figure 1 shows a typical rate experiment, in this case for PCE. A straight-line fit through the initial points was used to find the dechlorination rate, with the standard error of the slope being used to find 95% confidence intervals. The first measured CAH concentration was used as the initial concentration for this rate. The values kX and K_S were then calculated for each chlorinated ethene by minimizing the sum of the squares of the difference between the measured initial rate for a given concentration and that predicted by eq 1. Properties of the nonlinear least squares estimates of kX and K_S were analyzed by standard procedures (*20*) and are reported as 95% confidence intervals.

Results and Discussion

Batch Experiments. The measured initial dehalogenation rates for various CAH concentrations and best-fit curves by nonlinear least-squares fit to eq 1 are shown in Figure 2. The computed maximum degradation rates (kX) and half-velocity coefficients (K_S) along with their 95% confidence intervals are summarized in Table 2.

Maximum degradation rates depend on the concentration of dechlorinating organisms, which is some portion of the

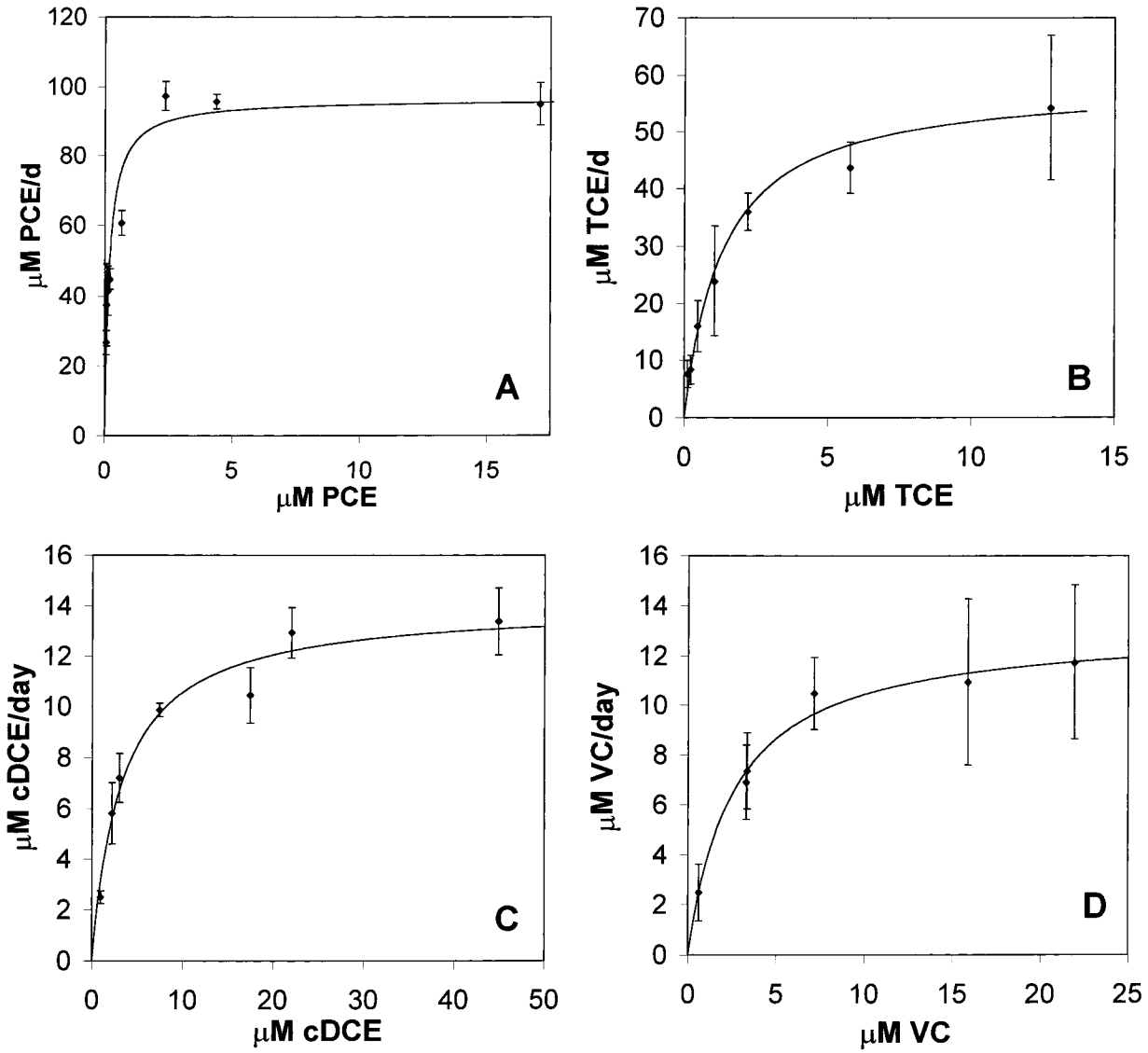

FIGURE 2. Initial dehalogenation rates for various initial concentrations of PCE (A), TCE (B), cDCE (C), and VC (D) using 38 mg/L VSS. Computed aqueous concentrations from headspace measurements are indicated. Filled diamonds and error bars reflect the best estimate and 95% confidence intervals, respectively, for the dehalogenation rate in each bottle. Curve is the best fit of best estimate values to eq 1 by nonlinear least squares.

TABLE 2. Half-Velocity Coefficients (K_S) and Maximum Aqueous Dechlorination Rates (kX) with Their 95% Confidence Intervals for Each of the Chlorinated Ethenes with 38 mg/L VSS[a]

	kX (μM/day)	k_{app} (μmol (mg of VSS)$^{-1}$ d^{-1})	K_S (μM)
PCE	77 ± 5	2.0 ± 0.1	0.11 ± 0.04
TCE	59 ± 11	1.6 ± 0.3	1.4 ± 0.9
cDCE	14 ± 3	0.37 ± 0.08	3.3 ± 2.2
VC	13 ± 3	0.34 ± 0.08	2.6 ± 1.9

[a] The apparent k (k_{app}) assuming X is equal to VSS is also provided.

total biomass measured as volatile suspended solids, and therefore varies widely from culture to culture. For comparison, the maximum dechlorination rates (kX) reported here are higher than in the sediment microcosms of Barrio-Lage et al. (*21*, *22*), comparable with the column results of deBruin et al. (*4*) and lower than the expanded-bed reactor findings of Carter and Jewell (*23*). These reports were for different total biomass concentrations, and the proportion of actual dechlorinators in each would no doubt depend on the feed type and level of enrichment. Similarly, the maximum dechlorination rate per milligram of biomass with our mixed culture is 1−2 orders of magnitude lower than has been reported for pure cultures or cell extracts (*7*, *8*, *24*, *25*). It should be noted that although VC is frequently reported by others to be degraded much slower than the other chlorinated ethenes (*15*), it was found here to be degraded at about the same rate as cDCE. However, PCE and TCE conversion rates were much higher than that for cDCE and VC, as commonly reported. Additionally, the conversion rates of the four CAHs in the CSTR (0.34 μmol (mg of VSS)$^{-1}$ d^{-1}) was about the same as the k_{app} for cDCE and VC but only about 20% of that for PCE and TCE. Thus, the CSTR had little additional capacity for cDCE and VC transformation but much more for PCE and TCE transformation. This is also in accordance with the generally observed rate differences between the more chlorinated and less chlorinated CAHs.

The half-velocity coefficients (K_S) measured here are an order of magnitude lower than Barrio-Lage estimated from their static microcosms (*21*, *22*), which are likely to have had severe mass transfer effects, but Carter and Jewell found the K_S for PCE (not measured for other chlorinated ethenes) in their expanded-bed reactor to be 0.054 ± 0.024 μM (*23*), which

is not greatly different than the 0.11 ± 0.04 μM found in this study. These half-velocity coefficients are much lower than the 200 μM reported for PCE and the 240 μM reported for TCE for the tetrachloroethylene reductive dehalogenase enzyme purified from *Dehalospirillum multivorans* (*26*), but they are comparable to the reported 10 μM for PCE and the 4 μM for TCE of a *Desulfitobacterium* sp. dehalogenase (*27*). The half-velocity coefficient for PCE was found in our culture to be significantly lower than that for the other chlorinated ethenes. Low concentrations of PCE are therefore likely to be quickly dechlorinated to TCE, which in turn would be degraded slower because of the much higher K_S.

The K_S values for TCE, cDCE, and VC are significantly above the maximum contaminant levels (MCLs) for these compounds of 0.038, 0.72, and 0.032 μM, respectively (*28*). For site cleanup, when the chlorinated ethene concentration levels drop into the low micromolar range of the MCLs, the dechlorination rate can thus be expected to decrease significantly from maximum values. Both the lower reaction rates (kX) and the higher K_S values can contribute to the frequent finding in the field of incomplete dehalogenation of PCE to ethene from intrinsic or engineered bioremediation. Also, in situ hydrogen concentrations, where dechlorination is active, are often as low as 2−10 nM, well below the reported K_S values for hydrogen electron donor of 20−100 nM (*13*, *14*). Thus, the combined impact of low electron donor and low electron acceptor concentrations relative to their respective K_S values could have an even greater impact on overall dehalogenation rates. For this reason when dehalogenation rates are being estimated, the limitations imposed by low concentrations of both the donor and the CAH should be considered.

Also, it should be noted that first-order kinetics are often assumed in numerical models of anaerobic dehalogenation. The results here suggest that zero-order kinetics are more appropriate for concentrations above a few micromolar or above the low milligram per liter range. The best approach, however, is to use Monod kinetics for modeling of CAH fate.

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